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Role of the 5-lipooxygenase pathway in obstructive nephropathy

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Role of the 5-lipooxygenase pathway in obstructive nephropathy. Leukotrienes are products of the 5-lipooxygenase pathway of arachidonic acid metabolism that possess potent inflammatory properties. We examined the potential role of this pathway in the decrease in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) observed in rats after unilateral release of bilateral ureteral obstruction (BUO) of 24 hours duration. Isolated glomeruli from rats with BUO produced significantly greater amounts of leukotriene B_4 (LTB_4) than glomeruli from sham-operated rats (SOR; $P < 0.0001$). Glomeruli from rats with BUO given MK886, an inhibitor of the 5-lipooxygenase enzyme, or from rats with BUO subjected to both total body irradiation to prevent the leukocyte infiltration of the kidney and also given MK886 prior to obstruction, produced amounts of LTB_4 not significantly different from those in glomeruli of SOR. Glomeruli from rats with BUO that had only total body irradiation prior to obstruction produced significantly less LTB_4 than glomeruli from untreated BUO rats, but LTB_4 production was still significantly greater than in glomeruli from SOR. There were no significant differences in GFR among SOR, SOR given MK886, and SOR subjected to total body irradiation. However, SOR given MK886 had significantly higher ERPF and lower renal vascular resistance (RVR) than SOR not pretreated with the lipooxygenase inhibitor. Rats with BUO given MK886, or subjected to total body irradiation, or both, prior to obstruction had significantly greater GFR and ERPF values and lower RVR than untreated BUO rats. Glomeruli from rats with BUO which were not pretreated had three times the leukocytes of glomeruli from SOR. This leukocyte infiltrate was composed of macrophages (about 55%) and neutrophils (about 45%). Pretreatment with the lipooxygenase inhibitor reduced the infiltrate by half, and total body irradiation practically abolished it. Cells infiltrating the kidney accounted for about two-thirds and intrinsic glomerular cells for one-third of the increase in leukotriene production in glomeruli from obstructed kidneys. This study indicates that increased synthesis of leukotrienes has a role in the hemodynamic changes seen after unilateral release of BUO of 24 hours duration.

Marked alterations in glomerular and tubular function occur after unilateral release of bilateral ureteral obstruction (BUO) of 24 hours duration [1]. The values for GFR and ERPF are approximately one-third of those obtained in a single kidney of sham-operated rats [2]. Products of arachidonic acid metabolism have a role in the observed hemodynamic changes. The activities of both cyclooxygenase and thromboxane synthase are increased in the kidney during BUO [3–5], and the glomerular production [6] and urine excretion [7] of the metabolites of the arachidonic pathway are greater in rats with BUO than in sham-operated rats. Increased glomerular synthesis of these products appears to be due, at least in part, to macrophages that infiltrate the kidney after the onset of obstruction [8]. Eicosanoids, products of arachidonic acid metabolism, play an important role in the pathophysiology of different models of inflammatory [9, 10] and noninflammatory renal disease [11]. Interestingly, total body irradiation, a maneuver that prevents leukocyte infiltration of the kidney, decreases the excretion of eicosanoids in the urine of rats with BUO [7].

In the present study we examined the role of the 5-lipooxygenase pathway of arachidonic acid metabolism in the renal alterations observed after unilateral release of BUO of 24 hours duration. This metabolic pathway leads to the formation of leukotrienes, potent chemoattractant, inflammatory and vasoactive mediators synthesized mainly by neutrophils and macrophages [12]. Leukotriene B_4 (LTB_4) predominantly induces leukocyte degranulation, superoxide generation and natural killer cell activity. Leukotrienes C_4, D_4 and E_4 constitute the slow reacting substance active in anaphylaxis. At the present time, LTB_4 is the only leukotriene for which the production has been detected and quantified in isolated glomeruli of the rat. We used three approaches to define the role of leukotrienes in rats with BUO. First, we measured the production of LTB_4, as an index of the activity of the 5-lipooxygenase, in glomeruli obtained from sham-operated rats and from rats with unilaterally released BUO of 24 hours duration. Second, we measured GFR and ERPF after unilateral release of BUO in rats given or not given MK886, an inhibitor of the translocation of 5-lipooxygenase from its inactive cytosolic location to its membrane-bound active location. This results in a physiologically inactive 5-lipooxygenase [13–16]. Third, we examined the contribution of invading leukocytes both to the glomerular production of leukotrienes, as measured by LTB_4, and to the effects of prior total body irradiation, alone or in combination with MK886 pretreatment, on renal function after unilateral release of BUO. Total body irradiation effectively blunts the leukocyte infiltration of the kidney seen after BUO of 24 hours duration [7]. Our results suggest that activation of the 5-lipooxygenase pathway of arachidonic acid metabolism results in increased synthesis of leukotrienes during BUO. These compounds, in turn, affect renal hemodynamics. The increased production of leukotrienes in glomeruli of rats with BUO of 24 hours duration correlates

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with the infiltration of the kidney by macrophages and neutrophils.

Methods

Animals and chemicals

Female Sprague-Dawley rats, weighing 165 to 248 g (mean 211.3 ± 5.1 g), were obtained from Sasco Inc. (Omaha, Nebraska, USA). After arrival the animals were housed five or six to a cage and were fed a standard rat chow containing 22.8% protein (Ralston Purina, St. Louis, Missouri, USA) and had water *ad libitum*. Animals were housed in our facilities for at least seven days prior to study.

The compound MK886 (3-[1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), an inhibitor of the translocation of 5-lipooxygenase from its inactive cytosolic location to its membrane-bound active location, was from Dr. Ford-Hutchinson (Pointe Claire-Dorval, Merck and Frosst, Quebec, Canada). This compound was prepared for oral administration to rats according to the manufacturer's recommendations: 20 μl of pre-warmed ethanol and 100 μl of 0.1% methylcellulose (Sigma Chemical Co., St. Louis, Missouri, USA) was added to 10 mg of MK886 [13]. This solution was gently heated until solubility occurred, and then 0.1% methylcellulose was added to yield 1 ml. This compound was administered by a stomach tube (Perfektum, New Hyde Park, New York, USA) at a dose of 10 mg/kg body wt four hours before induction of BUO and 12 hours before unilateral release of BUO. Inulin was purchased from Sigma Chemical Co. and *para*-aminohippuric acid (PAH) was purchased from Merck, Sharp & Dohme (West Point, Pennsylvania, USA).

Isolation of glomeruli

This procedure was performed as described previously [17]. Under light anesthesia with intraperitoneal sodium pentobarbital (50 mg/kg body wt; Nembutal®, Abbot Laboratories, Abbot Park, Illinois, USA), rats had an aortic clamp placed above the renal arteries. The aorta was cannulated at the bifurcation and perfused with 50 ml of pre-warmed normal saline until blanching of the kidneys was observed. At this time the kidneys were removed and cut sagittally, and the cortex was separated from the medulla. Glomeruli were obtained by passing the homogenized renal cortex through sieves of successively smaller diameters. Glomeruli were counted and permeabilized with digestion media containing collagenase type II (Worthington Biochemical Corp, Freehold, New Jersey, USA) and DNAase type I (Sigma) as described to preserve viability and maintain agonist responses [18, 19]. This method resulted in isolated glomeruli completely devoid of contaminating circulating cells. Glomeruli were subsequently incubated in Krebs-Henseleit buffer (approximately 30×10^3 glomeruli per 0.4 ml) for sequential 10-minute periods. After two incubations to stabilize the preparation, glomeruli were washed three times and a basal period of LTB_4 production was obtained. Glomeruli were then exposed to 10 μM ionomycin. Glomerular supernatants were assayed for LTB_4 content by a specific radioimmunoassay detailed previously [17]. The values were normalized for the number of glomeruli in the incubation and are expressed as fmol/10^3 glomeruli.

Bilateral ureteral obstruction and sham-operation

These procedures were performed as described previously [18]. Briefly, BUO was performed in rats under ether anesthesia by ligating both ureters at the junction of their lower third and upper two-thirds through a small suprapubic incision. Sham-operation consisted of opening the abdominal cavity of rats and manipulating but not obstructing both ureters. Both groups of rats were returned to their cages and were allowed water but no food prior to study, 24 hours later.

Clearance studies and blood pressure determinations

Standard clearance measurements were obtained as described previously [9]. Briefly, with animals under light ether anesthesia, catheters were inserted in the left femoral artery (PE 50), left femoral vein (PE 10) and left ureter (PE 50). The rats were secured in plastic holders, and two hours after recovery from anesthesia were studied in the awake state. A priming dose of chemical inulin designed to produce plasma levels of 70 to 150 mg/dl and chemical PAH calculated to produce plasma levels of 1 to 2 mg/dl was infused in 1 ml of normal saline over a three minute period. This was followed by a sustained infusion delivered at 40 μl/min, which contained sufficient inulin and PAH to maintain constant plasma levels of these compounds. After an equilibration period of at least 60 minutes and (in rats that had sustained BUO) approximately four hours after unilateral release of BUO, three consecutive 20-minute collections of urine and blood were obtained for estimation of GFR by inulin clearance (C_{in}) and ERPF by PAH clearance (C_{PAH}). Mean arterial pressure (MAP) was recorded throughout the experiment by means of the femoral artery catheter connected to a Harvard Apparatus (WECO VT-1, Winston Electronics Co, Millbrae, California, USA).

Total body irradiation

This procedure was performed in 15 rats as described previously [7]. Forty-eight hours prior to induction of BUO or sham-operation (72 hr prior to clearance studies and glomeruli harvesting) the rats were anesthetized with sodium pentobarbital (50 mg/kg body wt) and received whole body irradiation of 1315 rads over 10 minutes (Gamma cell 40, Atomic Energy of Canada Ltd), with shielding of the renal area.

Glomerular leukocyte quantification

Total leukocyte counts in permeabilized glomeruli were performed using a double antibody fluorescence technique as described previously [20, 21]. Mouse anti-rat leukocyte common mAb antibody (50 μg/ml) was used as the primary antibody followed by fluorescein-conjugated rabbit anti-mouse antibody (100 μg/ml). The labeled cell content of isolated glomeruli was evaluated by immunofluorescence microscopy with a Universal microscope (Carl Zeiss, Thornwood, New York, USA). Cells were focused through the glomerulus and counted as they appeared in the plane of focus. In each experiment, 50 to 100 glomeruli were counted.

To perform differential counts, glomeruli were dissociated into a single cell suspension by means of a previously described enzymatic protocol [20]. Leukocytes were then stained with an immunoperoxidase method using the same primary antibody as above and the Zymed histostain kit from Chemicon (Temelula,

California, USA). Cells were pelleted onto glass slides by cytocentrifuge (Shannon, Astmore, UK), fixed in acetone, air-dried, developed for immunoperoxidase and counterstained with hematoxylin (Baxter). Positively labeled cells were visualized by light microscopy and categorized by nuclear morphology.

Experimental groups

Twelve groups of rats were studied. Rats sustaining 24 hour BUO were studied immediately after unilateral release of the obstruction; other animals were studied 24 hours after sham operation. Groups 1 to 5 were used for isolation of glomeruli and determination of LTB_4 production. Group 1 consisted of six sham-operated rats (SOR). Group 2 consisted of nine rats with BUO of 24 hours duration. Group 3 consisted of six rats with BUO given MK886 by gastric lavage (10 mg/kg body wt 4 hr before BUO and 12 hr before unilateral release of BUO). Group 4 consisted of six rats that had total body irradiation 48 hours prior to BUO. Group 5 consisted of five rats that received total body irradiation 48 hours prior to BUO and in addition were given two doses of MK886 as in group 3. Groups 6 to 8 were used to study renal function in SOR. Group 6 consisted of five SOR that served as temporal controls. Group 7 consisted of four rats that were pretreated with MK886 as described above. Group 8 consisted of three rats that received total body irradiation 72 hours prior to studies. Groups 9 to 12 were used to study renal function after unilateral release of BUO of 24 hours duration. Group 9 consisted of six rats with BUO pretreated with the vehicle used to dissolve the MK886. Group 10 consisted of eight rats with BUO pretreated with MK886 as described above. Group 11 consisted of four rats that received total body irradiation 48 hours prior to BUO. Group 12 consisted of five rats that received total body irradiation 48 hours prior to BUO and were given MK886 as in group 3.

Leukotriene B_4 measurements and other analytic determinations

The production of LTB_4 from isolated glomeruli obtained from rats of groups 1 to 5 was measured by radioimmunoassay as described previously [18–20]. Sodium and potassium were measured by standard flame photometry (Instrumentation Laboratory Inc, Lexington, Massachusetts, USA). Inulin was determined using the anthrone method [22] and PAH was measured by a modification of the method of Smith et al [23].

Calculations

Clearance values for inulin and PAH were calculated using standard formulas. Renal vascular resistance (RVR) was calculated according to the formula MAP/ERPF(1-hematocrit). For each rat the values of three consecutive clearance periods were averaged. Analysis of variance was used for comparisons among different groups for the several determinations performed. Differences were considered significant when $P < 0.05$.

Results

Figure 1 depicts the production of LTB_4 in isolated glomeruli from sham-operated rats (group 1), rats with BUO (group 2), rats with BUO given MK886 (group 3), rats subjected to total body irradiation prior to BUO (group 4) and rats subjected to

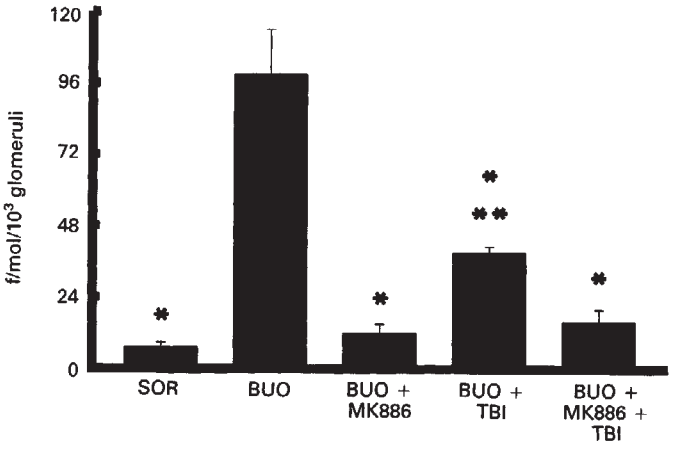

Fig. 1. *Production of leukotriene B_4 by isolated glomeruli obtained from sham-operated rats (SOR), rats with bilateral ureteral obstruction (BUO), rats with bilateral ureteral obstruction pretreated with the inhibitor of the 5-lipooxygenase MK886 (BUO + MK886), rats with bilateral ureteral obstruction subjected to total body irradiation (BUO + TBI), and rats subjected to both total body irradiation and administration of MK886 prior to obstruction (BUO + TBI + MK886).* Glomeruli obtained from rats with BUO produced significantly greater amounts of leukotriene B_4 than glomeruli from SOR ($*P < 0.01$). It is of note that all of the maneuvers that inhibited lipooxygenase activity (MK886) or prevented the leukocyte infiltration of the kidney (TBI) resulted in a marked and significant decrease in the production of leukotriene B_4 by isolated glomeruli ($*P < 0.01$). Glomeruli obtained from rats with BUO subjected to total body irradiation prior to obstruction produced significantly greater amounts of leukotriene B_4 than glomeruli from SOR ($**P < 0.01$).

total body irradiation which also received MK886 prior to BUO (group 5). Glomeruli from rats with BUO produced significantly more LTB_4 (99.0 ± 15.0 fmol/10^3 glomeruli) after stimulation with ionomycin than isolated glomeruli obtained from sham-operated rats (7.3 ± 1.6 fmol/10^3 glomeruli). The different maneuvers employed decreased significantly the production of LTB_4 in glomeruli of rats with BUO (groups 3 to 5) compared to untreated BUO rats. The production of LTB_4 in glomeruli of rats with BUO given MK886 (12.0 ± 3.0 fmol/10^3 glomeruli) or given MK886 and also subjected to total body irradiation (16.0 ± 4.0 fmol/10^3 glomeruli) was not significantly different from the production of LTB_4 in glomeruli from SOR. The production of LTB_4 in glomeruli obtained from BUO rats subjected only to total body irradiation was significantly lower (39.0 ± 2.0 fmol/10^3 glomeruli) than that obtained in untreated BUO rats. However, this value was significantly greater than that obtained in glomeruli from SOR.

To determine the effects on renal function of inhibiting 5-lipooxygenase in the setting of obstruction, we obtained standard clearance measurements in SOR (groups 6 and 7) and rats with BUO (groups 9 to 12) in which different maneuvers were used to modify the production of leukotrienes. There were no significant differences in body weight, urine flow rates, fractional sodium excretion or fractional water excretion between the two sham-operated groups (Table 1). However, absolute sodium excretion was significantly lower in SOR given MK886 than in temporal controls. There were no significant differences in body weight among the different groups of rats with BUO (Table 1). Absolute sodium excretion, but not

Table 1. Body weights, absolute and fractional sodium and water excretion in two groups of SOR and four groups of rats with BUO in which renal function was measured

	Group	Body weight *g*	Urine flow *μl/min*	UV_{Na} *μEq/min*	FE_{Na} %	FE_{H_2O} %
Sham-operated rats						
Temporal controls	6	219 ± 6	18.7 ± 5.5	5.8 ± 1.3	0.8 ± 0.1	2.4 ± 0.6
SOR + MK886	7	220 ± 5	13.1 ± 3.0	1.8 ± 0.6[a]	0.98 ± 0.31	0.9 ± 0.2
Rats with BUO						
Temporal controls	9	193 ± 5	35.5 ± 5.2	3.6 ± 0.7	9.8 ± 1.8	13.8 ± 1.5
BUO + MK886	10	214 ± 9	38.5 ± 3.0	3.6 ± 0.5	3.9 ± 0.7[a]	6.3 ± 0.9[a]
BUO + TBI	11	203 ± 3	51.8 ± 8.5	5.4 ± 1.4	5.6 ± 1.4[a]	8.3 ± 1.3[a]
BUO + MK886 + TBI	12	207 ± 6	66.8 ± 6.1[a]	8.0 ± 0.6[a]	8.1 ± 0.7	13.4 ± 0.7

Abbreviations are: SOR, sham-operated rats; BUO, bilateral ureteral obstruction; TBI, total body irradiation; UV_{Na}, absolute sodium urine excretion; FE_{Na}, fractional sodium excretion; FE_{H_2O}, free water clearance.
[a] $P < 0.05$ when compared to temporal controls

Table 2. Renal function, mean arterial blood pressure and renal vascular resistance in sham-operated rats and rats with bilateral ureteral obstruction

	Group	GFR *ml/min/kg*	ERPF *ml/min/kg*	MAP *mm Hg*	RVR *mm Hg/ml/min/kg*
Sham-operated rats					
Temporal controls	6	6.37 ± 0.33	21.5 ± 0.8	119 ± 1	3.15 ± 0.13
SOR + MK886	7	6.63 ± 0.53	28.9 ± 1.5[a]	123 ± 2	1.26 ± 0.06[a]
SOR + TBI	8	6.80 ± 0.47	ND	120 ± 1	ND
Rats with BUO					
Temporal controls	9	1.37 ± 0.14	2.38 ± 0.17	158 ± 5	37.0 ± 2.9
BUO + MK886	10	3.15 ± 0.32[a]	11.4 ± 1.0[a]	153 ± 4	8.2 ± 0.8[a]
BUO + TBI	11	3.33 ± 0.73[a]	7.77 ± 0.23[a]	164 ± 5	8.4 ± 1.9[a]
BUO + MK886 + TBI	12	3.16 ± 0.27[a]	7.99 ± 1.17[a]	149 ± 8	10.5 ± 3.2[a]

Abbreviations are: SOR, sham-operated rats; BUO, bilateral ureteral obstruction; MAP, mean arterial pressure; TBI, total body irradiation; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; RVR, renal vascular resistance; ND, not determined.
[a] $P < 0.05$ when compared to temporal controls

fractional sodium or fractional water excretion, was significantly greater in rats of group 12 than in untreated BUO rats (group 9). In contrast, rats with BUO given MK886 (group 10) or subjected to total body irradiation (group 11) had significantly lower fractional sodium and fractional water excretion than untreated BUO rats (group 9). Table 1 shows that rats of group 12 had significantly greater urine flow than temporal controls.

Table 2 summarizes the values for GFR, as measured by C_{in}, ERPF, as measured by C_{PAH}, MAP and RVR obtained in SOR (groups 6 to 8) and rats with BUO (groups 9 to 12). Untreated BUO rats (group 9) had a significantly lower GFR (about 80% lower), ERPF (about 90% lower) and higher MAP (about 33% increase) and RVR (about a 12-fold increase) than SOR that served as temporal controls (group 6). There were no significant differences in GFR or MAP among the different groups of SOR. However, rats with SOR given MK886 (group 7) had significantly higher ERPF and lower RVR than untreated SOR (group 6). Due to a laboratory accident, it was not possible to determine ERPF or RVR in SOR subjected to total body irradiation (group 8). There were no significant differences in MAP among the different groups of rats with BUO. Despite this, the different maneuvers used to decrease leukotriene production in these rats (groups 10 to 12) resulted in significantly higher values for GFR and ERPF and significantly lower values for RVR than the ones obtained in untreated BUO rats (group 9). Although rats with BUO given MK886 (group 10) tended to have higher values for ERPF than the other BUO groups tested, the values for RVR were not significantly different from those observed in rats with BUO undergoing total body irradiation (group 11) or rats with BUO subjected to total body irradiation and given MK886 (group 12).

Figure 2 summarizes the results of total leukocyte counts in glomeruli obtained from SOR and BUO rats. Glomeruli obtained from SOR had fewer leukocytes than glomeruli from untreated BUO rats. Interestingly, the infiltrate in the glomeruli from untreated BUO rats was mixed and composed not only of macrophages (54%) but also of neutrophils (46%), and to the best of our knowledge this constitutes the first demonstration of a role for neutrophils as invaders of the glomerulus in the setting of BUO in the rat. In glomeruli obtained from rats given MK886 prior to BUO of 24 hours duration the leukocyte infiltrate was reduced by about one-half. Prior irradiation of rats that had BUO almost completely prevented the leukocyte infiltration of the glomeruli.

Discussion

The results of this study demonstrate a significant increase in the glomerular production of LTB_4 after BUO of 24 hours duration. This increase was blunted by the administration in

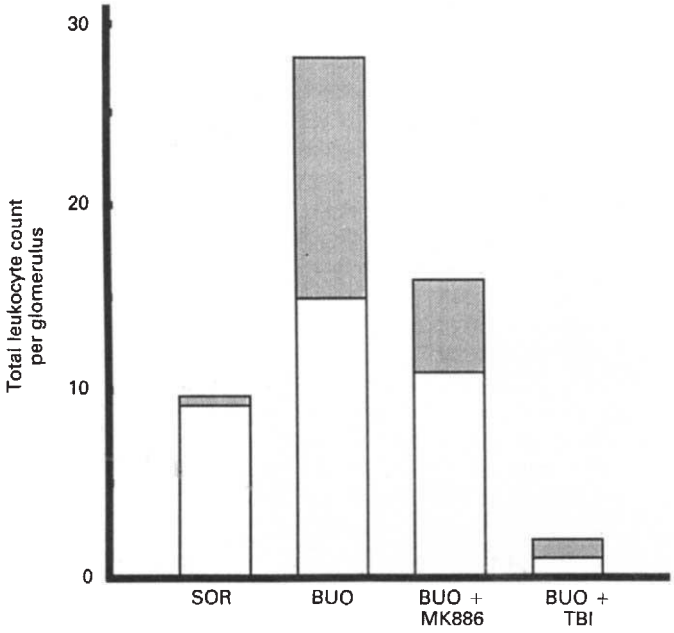

Fig. 2. *Leukocytes present in glomeruli of sham-operated rats (SOR) or rats with bilateral ureteral obstruction (BUO).* Rats with BUO had a greater number of leukocytes per glomerulus than sham-operated rats (SOR), and the infiltrate consisted of macrophages (□) and neutrophils (▨). Neutrophils were practically absent in glomeruli from SOR. BUO rats pretreated with MK886, the inhibitor of 5-lipooxygenase, showed a marked decrease in the glomerular infiltrate by leukocytes. In rats subjected to total body irradiation (TBI) prior to BUO the leukocyte infiltrate was almost completely abrogated.

vivo of an inhibitor of 5-lipooxygenase prior to obstruction and isolation of glomeruli, by total body irradiation prior to BUO, or by the combination of these two maneuvers. At the same time the renal functional studies demonstrated that inhibition of 5-lipooxygenase with MK886 or prevention of the leukocyte infiltrate in the kidney during obstruction with either MK886 or total body irradiation ameliorated the decrease in GFR and ERPF observed after unilateral release of bilateral ureteral obstruction. The greater values for GFR and ERPF after 5-lipooxygenase inhibition or prevention of the leukocyte infiltration were not accompanied by marked changes in systemic blood pressure, suggesting that these maneuvers very likely modified intrarenal hemodynamics. In fact, they resulted in significant reductions in renal vascular resistance to about one-fourth of the values obtained in untreated BUO rats (group 9). However, the present studies do not allow us to determine whether the decrease in GFR was due to a fall in the ultrafiltration coefficient (K_f), a fall in intraglomerular pressure or a combination of both.

The tubuloglomerular balance of the different groups of rats after release of BUO deserves some comment. Values for GFR after release of BUO in temporal controls were about one-fifth those of untreated SOR (Table 2) and urine flow values were about twofold those obtained per one kidney in untreated SOR (Table 1). Fractional sodium and water excretions were markedly increased after release of BUO of 24 hours duration. The basis for this postobstructive diuresis is not completely understood, but factors such as resistance of distal tubular segments to vasopressin [24], an osmotic diuresis [25] and increased levels of atrial natriuretic peptide [26] may contribute to its occurrence. In the present study, rats given MK886 prior to BUO had fractional sodium and water excretions that were lower than the values in untreated BUO rats (group 9). This is probably due to the greater filtered load of sodium and water as a consequence of the increase in GFR. Rats subjected to total body irradiation prior to BUO had urine flow values and fractional excretions of sodium and water that were greater than in BUO rats given MK886. This occurred despite comparable filtered loads of sodium and water. In rats subjected to total body irradiation and also given MK886 prior to BUO, the filtered load of sodium and water was twice as great as in rats with BUO not treated. However, the fractional excretion of sodium and water did not differ from that of untreated BUO rats (group 9). This suggests a potential diuretic and natriuretic effect brought about by the irradiation treatment. The mechanisms underlying this effect are not readily apparent.

Measurements of LTB_4 were performed in isolated glomeruli that were perfused with normal saline and devoid of circulating cells. This method of glomeruli isolation has been shown to result in glomeruli that only contain resident macrophages and other cells that have previously invaded the kidney [17]. The present results are in agreement with previous reports of the ability of glomeruli to synthesize leukotrienes after immunological injury to the kidneys. Schreiner and Lefkowith in the nephrotoxic-induced serum nephritis model (NSN) [17, 19, 20], Lianos in the passive Heymann nephritis and the NSN model [11, 27] and Rahman et al in cationic gamma globulin-induced glomerulonephritis [28] have demonstrated increased production of LTB_4 by isolated glomeruli. The increased production of LTB_4 observed in isolated glomeruli obtained from rats with BUO correlates with the inflammatory infiltrate observed after ureteral obstruction of 24 hours duration. Thus, the increased production of LTB_4 by isolated glomeruli may be in part the result of leukocyte infiltration. After BUO of 24 hours duration we found a significant leukocyte infiltration in the kidney, composed mainly of macrophages and neutrophils. This leukocyte invasion peaks between 12 and 24 hours after the onset of obstruction [8] and its onset at four hours after obstruction is temporally related to the initial decrease in ERPF, indicating active production (or modulation) of vasoconstrictor substances by these cells. Abrogation of the infiltrate by total body irradiation or its significant reduction by MK886 ameliorated the decrease in GFR and ERPF observed after unilateral release of BUO, and was correlated with decreased production of LTB_4 by isolated glomeruli. This suggests that the invasion of the kidney by macrophages and neutrophils after BUO significantly increased the ability of glomeruli to synthesize leukotrienes and affected renal hemodynamics. Glomeruli obtained from rats that were given MK886 prior to BUO showed a 90% reduction in the synthesis of leukotrienes, as monitored by LTB_4 production compared to untreated BUO rats. Complete abrogation of the leukocyte infiltrate by total body irradiation reduced production of LTB_4 to 60% of the values seen in untreated BUO rats. On the other hand, partial reduction of the leukocyte infiltrate with MK886 decreased the production of LTB_4 to levels similar to those obtained in sham-operated rats. These observations suggest that cells intrinsic to the glomerulus account for about one-third of the total increase in leukotriene

production found in this setting. All the increase in leukotriene production seen after BUO of 24 hours duration was completely abolished by an inhibitor of the activation of 5-lipooxygenase. This suggests that the inhibitor affected LTB_4 production in both intrinsic glomerular cells and invading leukocytes.

Studies designed to examine the effect of inhibition of 5-lipooxygenase with MK886 on the leukocyte infiltrate of glomeruli revealed a decrease of approximately 50% in the leukocyte infiltrate in treated BUO rats compared to values observed in untreated BUO rats (Fig. 2). Thus, inhibition of 5-lipooxygenase in rats with BUO resulted in: 1) reduction in the glomerular production of leukotrienes, as assessed by LTB_4 measurements; 2) glomerular decrease in the leukocyte infiltrate; and, 3) significant amelioration of the decrease in GFR and ERPF. The fact that the inhibition of leukotriene synthesis in rats with BUO partially prevented the macrophage and neutrophil infiltration suggests that a product of the lipooxygenase pathway of arachidonic acid metabolism may be a key chemotactic factor for these cells during obstruction of the urinary tract. The improved renal hemodynamics seen in rats with BUO given MK886 may be due to the combined effects of decreasing the macrophage infiltrate and to a decrease in leukotriene production by circulating and/or intrinsic cells of the kidney. These leukotrienes were probably those with vasoactive properties of the C_4, or D_4 series. In the present studies the glomerular production of other metabolites of arachidonic acid such as the cysteinil-containing leukotrienes C_4, D_4 and E_4 was not detectable with specific radioimmunoassay techniques (data not shown), suggesting that such levels are very low and/or that these leukotrienes are synthesized only by circulating cells. It is possible that the observed beneficial effect of 5-lipooxygenase inhibition on renal hemodynamics could be due to decreased synthesis of leukotrienes with vasoconstrictor properties such as leukotrienes of the C_4 and D_4 series, and the prevention of a leukocyte infiltrate in the glomeruli. Such leukocytes, mainly macrophages, are capable of releasing thromboxane A_2, another vasoconstrictor that contributes to the renal hemodynamic changes [7].

Studies by Badr et al [29, 30] have demonstrated that leukotrienes affect renal hemodynamics. In anesthetized rats infusion of LTC_4 produced a significant increase in MAP and a decrease in renal plasma flow [29]. Micropuncture studies demonstrated that LTD_4 reduced glomerular plasma flow and the ultrafiltration coefficient (K_f) [30]. In addition, in vitro studies suggest that the decrease of K_f observed after administration of LTC_4 and D_4 in vivo may be due to mesangial cell contraction with a decrease in the surface area available for filtration [31, 32]. Our studies in rats with BUO that had received total body irradiation and/or MK886 demonstrated amelioration of the decrease in ERPF and GFR without significant changes in systemic blood pressure, and this improvement was accompanied by a significant decrease in renal vascular resistance. Whether this decrease was associated with an increase in intraglomerular pressure and/or an increase in K_f could not be determined from the present studies.

In summary, we report a significant increase in the production of leukotrienes, as determined by LTB_4 synthesis, in isolated glomeruli obtained from rats with BUO of 24 hours duration, suggesting an increase in the biological activity of the 5-lipooxygenase pathway of arachidonic acid metabolism. Prior inhibition of the activation of this enzyme or prevention of the macrophage and neutrophil infiltrate to the kidney by total body irradiation or MK886 administration ameliorated the decrease in GFR and ERPF seen after unilateral release of BUO of 24 hours duration. This suggests an increased synthesis of metabolites of this pathway with chemoattractant properties (LTB_4), but also very likely other metabolites with vasoconstrictive activity (leukotrienes C_4 and D_4). Inhibition of 5-lipooxygenase with MK886 or pretreatment with total body irradiation decreased the synthesis of leukotriene in glomeruli and prevented the macrophage and neutrophil infiltrate. This suggests that a product(s) of the 5-lipooxygenase pathway of arachidonic acid metabolism may be a chemotactic factor for the leukocyte infiltration of glomeruli. Although the infiltrating leukocytes appear to be an important source of increased leukotriene synthesis in glomeruli, resident glomerular cells account for at least one-third of the production of leukotrienes after BUO of 24 hours duration. We conclude that increased synthesis of leukotrienes may play an important role in the altered hemodynamics seen after unilateral release of BUO of 24 hours duration.

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